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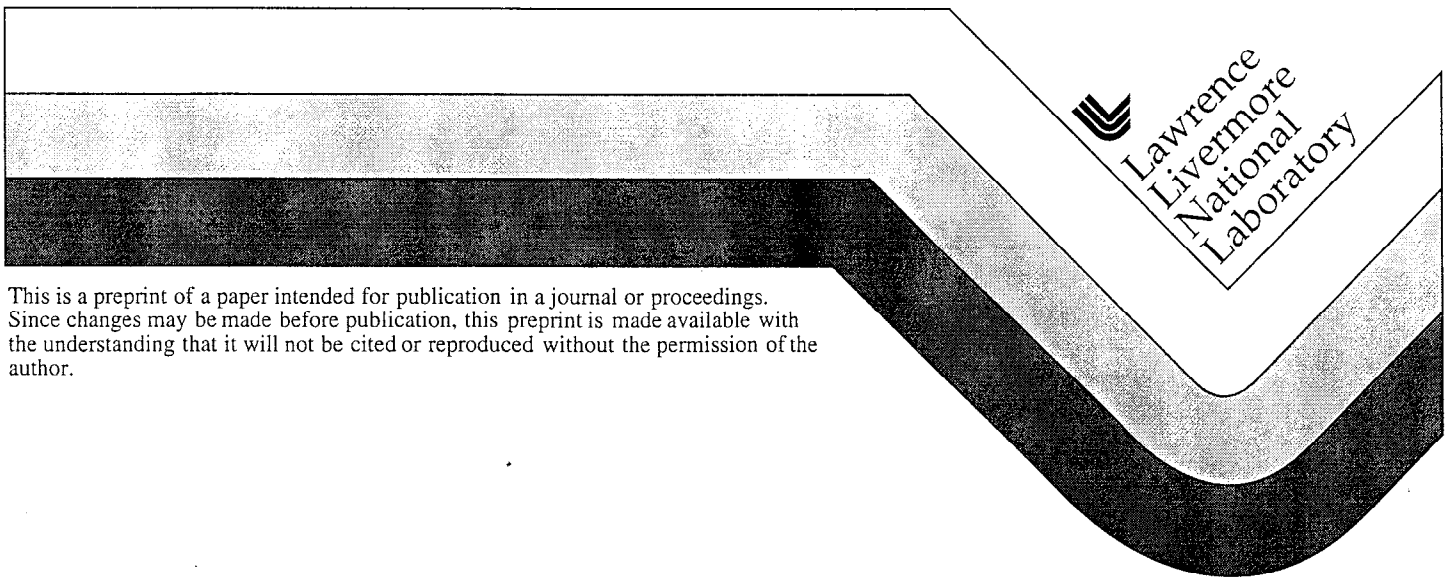

APPLICATION OF MOLECULAR CYTOGENETIC METHODS TO BIOLOGICAL DOSIMETRY

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The advent of molecular cytogenetic methods to quantify chromosome translocations has made these events the hallmark of radiation biological dosimetry. Translocations survive mitosis and involve minimal loss of DNA, in contrast to dicentrics which cause cell lethality. The key assumptions for retrospective dosimetry are that translocations persist and accumulate, thus making them ideal candidates for assessing acute and chronic exposures. This chapter summarizes the principles that are important for retrospective human exposure analysis, and presents and discusses recent data relevant to each principle. Observations to date indicate that translocation frequencies are not as constant as once believed. We have shown that translocation frequencies decline with time following acute radiation exposure and eventually reach a dose-dependent plateau. Translocations accumulate with chronic exposure, and increase with age in unexposed people. Cigarette smoking has also been shown to confound radiation exposure assessments. Expansion of clones of abnormal cells has been observed in both exposed and unexposed people, and can be expected to provide skewed exposure estimates. However, exposure assessments using translocations can provide meaningful and quantitative retrospective biodosimetry many years after acute or chronic radiation exposure provided the basic principles outlined here are incorporated into the study design.

1 INTRODUCTION

Structural chromosome aberrations have been studied for more than 50 years and thousands of papers have been published on the effects of DNA damaging agents in inducing cytogenetic damage. At the molecular level, aberrations are believed to result from double strand breaks in DNA that remain unrepaired or that undergo aberrant rejoining, giving rise to chromosomal rearrangements. While dozens of physical, chemical, and biological agents induce DNA damage, ionizing radiation is among the most efficient in inducing double strand breaks that lead to the formation of chromosome aberrations. It has been known for more than three decades that radiation induces dose-dependent increases in asymmetrical (e.g., dicentrics) and symmetrical (e.g., translocations) aberrations in cells throughout the body, including mature cells and progenitor stem-cells in the hematopoietic lineage. Induced aberrations in human lymphocytes have been used as biological dosimeters to gauge exposure levels since the early 1960's. Information obtained from numerous radiation cytogenetic studies has provided insight into the mechanisms that govern the induction, accumulation, persistence, and elimination of chromosome damage and of the relevance of such damage to the risks of late effects

in exposed populations. Attempts to derive recommendations regarding the applicability of cytogenetic techniques in population epidemiology must take into account a wealth of information that has accumulated over the past 30 years. One of the compelling arguments for studying cytogenetics is the relationship between chromosome aberrations and tumor formation. Of particular interest is knowing whether aberration frequencies can be used to predict cancer risk. Several recent reports[1, 5] have shown that aberration frequencies are increased prior to the clinical manifestation of disease. If this relationship holds true upon further investigation, then it will be important to determine the statistical strength of this predictability for various types of cancer, and to decipher the biological mechanisms responsible for these intriguing observations.

The well-understood relationship between chromosome damage and exposure to ionizing radiation is the primary rationale for using cytogenetic methods to quantify adverse exposure to radiation. In the early years biodosimetry was achieved by scoring dicentric and ring chromosomes. However, when cytogenetic analyses take place many months or years after the incident, as occurred with the A-bomb survivors and to a more limited extent with other exposures, the frequency of dicentrics and rings and hence the ability to obtain optimal biodosimetry is significantly diminished. The reason is that these aberrations undergo negative selection during cell division, and thus they have limited utility following chronic, low-level exposure.

Biodosimetry for temporally displaced or chronic exposure is improved substantially by examining translocations because cells with these events are known to persist through mitosis and to remain viable for many years. Fluorescence *in situ* hybridization with whole chromosome paints allows the selective identification of one or more pairs of chromosomes and enables recognition of breakage and exchange events between painted and non-painted chromosomes. Importantly, the frequencies of structural chromosome aberrations identified by painting have been validated by comparison with more established techniques and shown to yield equivalent results[23]. For this reason plus the rapid and precise identification of translocations, chromosome painting has now been established as the method of choice for retrospective biological dosimetry[14, 15, 25].

2 PARAMETERS FOR RADIATION EXPOSURE ASSESSMENT

The parameters important for understanding the association between adverse exposure and chromosome damage are induction, persistence and accumulation.

Induction is the measure of the relationship between exposure (dose) and the resulting biological response. The induction of chromosome aberrations is typically evaluated shortly after exposure to ensure that the peak level of initial

damage is quantified. Numerous dose-response curves have been published which define the relationship between aberrations and various types of ionizing radiation, and which describe the importance of factors such as dose fractionation. It is beyond the scope of this chapter to discuss this subject in detail.

Persistence is a measure of the longevity of induced damage. Until recently, cytogenetic measurements of persistence were hampered because translocation detection methods were laborious. Good measurements of the persistence of translocations following acute *in vivo* exposure were made possible by the development of chromosome painting probes for laboratory mice[20]. Similar assessments have been made following *in vitro* exposure to peripheral blood lymphocytes from rats[21] and humans[4, 12]. One of the key assumptions for retrospective biological dosimetry, described in more detail below, is that the kinetics of translocation persistence is known.

Accumulation is a measure of the total amount of damage in a cell, tissue, animal or person. For damage to accumulate, it must be induced and persist long enough to be measured. Until recently, few population studies have successfully measured the accumulation of chromosome aberrations because the types of damage events that were quantified were generally rare and lacked sufficient persistence for accumulation to occur. However, the use of chromosome painting has now provided very good evidence that cytogenetic damage in humans naturally accumulates, presumably as a result of daily exposure to chemicals in food, water, air, and terrestrial and extra-terrestrial ionizing radiation, and perhaps facilitated by diminished DNA repair associated with aging and inherited DNA repair capability[17, 22].

Translocations also have substantial utility for estimating chronic or highly fractionated radiation exposure. This is especially significant because most human exposures occur over a period of months or years. The ability of induced translocations to survive for prolonged periods of time in exposed individuals suggests that they can be used to measure chronic exposures. Some studies of translocations for biodosimetry in chronically exposed populations have been published[25], and the use of rodent models has yielded important insights into highly fractionated radiation exposures[24]. However, much more work needs to be done before there is a clear understanding of the factors involved in the accumulation of translocations with chronic exposure.

3 PRINCIPLES FOR RETROSPECTIVE EXPOSURE ANALYSIS

The inherent ability of cells with translocations allows them to survive mitosis and makes these aberrations ideal candidates for performing retrospective biological dosimetry. However, numerous factors are known or suspected from theoretical grounds to affect the induced translocation frequency, and these complicate the

process of estimating the true dose. To the extent that cells with translocations are persistent they are appropriate markers for evaluating prior exposure. If translocations lead to diminished cell survival, or if other factors not related to the exposure in question influence translocation frequencies, then the exposure assessments will be confounded. Thus, the use of painting to quantify translocations for retrospective biodosimetry involves a number of considerations, which are listed and discussed here.

3.1 Selection against cells damaged by the exposure does not occur or can be taken into account.

Of paramount importance to the use of translocations as a retrospective biomarker of exposure is the assumption that translocation frequencies remain constant with time, which stems from the belief that cells with translocations experience no selective pressure. This view is supported by several lines of evidence, including the existence of people in whom a reciprocal translocation exists in every cell and who are otherwise healthy (except for obvious reproductive risks), and laboratory animals that have carried certain translocations for multiple generations. However, none of these arguments supporting complete persistence of translocations have considered the possibility that a *portion* of radiation-induced translocations may undergo negative selection. In other words, just because *some* translocations may persist indefinitely does not mean they *all* will. For example, mice homozygous for translocations are well known to have phenotypic effects indicative of genetic damage, and humans heterozygous for translocations have been observed to have abnormal phenotypes. Evidence obtained *in vitro* on double strand break rejoining indicates that up to 8 kb of DNA may be deleted[11], which is large enough to carry a significant risk for disrupting an essential gene. Translocation breakpoints at (or very near) target gene sequences have been demonstrated cytogenetically, but their involvement has also been inferred from molecular analysis[13]. One radiation-induced translocation involving the hprt gene has been identified with cytogenetic and molecular methods[19]. Of significance here is the fact that the translocation was associated with a deletion of > 1 Mbp. Thus it is clear that translocation formation involves loss of genetic information, and that the selective pressure resulting from this loss not only remains uncertain but may vary widely.

Whether cells bearing radiation-induced translocations experience negative selection is currently a matter of much debate. Some laboratories have reported no change in translocation frequencies over time, while others have demonstrated clear temporal declines. Translocations measured in tumor cells shortly after *in vitro* irradiation showed no change in frequency over a three week period[8]. In rhesus monkeys, translocation frequencies induced *in vivo* were similar to frequencies of dicentrics induced acutely *in vitro*[10], suggesting that translocation frequencies had

remained unchanged over may years. An individual exposed to tritium has also been assessed[9] and translocation frequencies measured 11 years after exposure were no different than dicentrics scored 39 days after the accident. Others have shown that translocation frequencies in victims of the radiation accident in Goiania, Brazil were significantly lower six years after exposure than dicentrics measured immediately after the accident[15]. In our own work we have consistently observed a decline in translocation frequencies over time. *In vitro* experiments using human[12] or rat[21] whole blood indicate a loss of translocations of approximately 40%, presumably as a result of selection during cell division. Other investigators, however, did not observe such a loss[4]. A long-term follow-up experiment in mice acutely exposed to whole-body radiation at age 8 weeks clearly showed a decline in translocation frequencies out to one month following exposure[20], and unpublished data from this same experiment indicates that translocation frequencies continued to decline out to three months and were then stable for a year, after which clonal expansion of abnormal cells became significant. Similar results were observed in splenocytes of mice followed for more than 100 days after *in vivo* exposure to 2 Gy X rays[6]. Thus, there is good evidence that translocation frequencies decline with time, at least under some circumstances, and reach dose-dependent plateaus. Clearly, more work must be done to identify the conditions and parameters of the long-term persistence of translocation frequencies.

3.2 The translocation frequency pre-existing in the exposed individuals should be known or at least be estimated from appropriately matched controls.

This is an essential aspect of human exposure studies because translocations, like other biological endpoints, have a non-zero baseline frequency. The observation that translocation frequencies increase significantly with age[17], discussed in more detail below, emphasizes the importance of appropriately matched controls. Thus, the involvement of epidemiologists in the design and execution of population exposure assessments is of paramount importance.

3.3 Clones of cytologically abnormal cells are recognizable and the number of such clones, as well as their prevalence, can be accurately measured.

When proliferation of cytogenetically abnormal cells occurs to a greater or lesser extent than for normal cells, dose calculations may be over- or under-estimated accordingly[7]. Unrecognized clones will adversely affect the ability to enumerate the true level of induced translocation events (versus those actually observed). Clonal

expansion of damaged cells is not expected *a priori* to occur to a different extent than for normal cells, but stochastic processes that produce expanded or contracted clones in the cells studied must be kept in mind. Unfortunately, the methods used in most studies have not always made it possible to determine whether differential cell proliferation has occurred. Consequently most cytogenetic analyses of exposed populations have ignored the possibility of clonal expansion. Until the advent of chromosome painting, the frequency of clones bearing induced translocations in human peripheral blood lymphocytes was unknown. Clones of abnormal cells have now been observed in a number of exposed and control individuals, and in control individuals range in frequency up to ~1% of all metaphase cells[7]. As the use of multiple colors in chromosome painting becomes more routine, the ease with which clones can be recognized and enumerated will increase accordingly.

3.4 Breaks are distributed among chromosomes in a manner that is proportional to their size.

Chromosome breaks and rearrangements are theoretically expected to be distributed randomly throughout the genome. The assumption of random breakage is particularly important for chromosome painting because typically only a few of the chromosomes are labeled, and hence only a fraction of the total damage can be observed. This fraction is calculated based on chromosome size[21], and the results are extrapolated to the whole genome. If one or more of the painted chromosomes is not representative of the genome then the translocation frequency will be biased. Early data from banded human material showed that the correlation between chromosome DNA content and radiation-induced translocations is quite high, and that no one human chromosome accounts for substantially more or less than its share of the total number of translocations. This subject has also been addressed more recently[7]. A review of the literature supports the view that chromosome breakage and rearrangements generally occur randomly, except for the sex chromosomes which break less frequently than expected.

3.5 The rate of exposure is known, and the effects of dose rate upon translocation frequencies are understood.

The effects of radiation dose rate on chromosome aberration frequencies have been recognized for many years. Chronic or highly-fractionated radiation exposure produces markedly less chromosome damage than an equivalent acute exposure[24]. For chemicals there is also evidence for a dose rate effect. In studies of laboratory mice, chronic exposure to the clastogenic compounds cyclophosphamide and ethyl carbamate did not induce translocations[2]. However, separate experiments involving

acute exposures to these same compounds produced measurable cytogenetic damage, suggesting that chronic chemical exposure does not result in a sufficient number of double strand breaks that are close enough together in space and time to permit the formation of translocations.

3.6 The influence of other confounding exposures, which may fluctuate with time, are negligible.

This assumption is violated routinely because of the dynamic nature of human behavior. The primary and perhaps most significant example is cigarette smoking which is known to vary in intensity, particularly when smokers attempt to quit. Cigarette smoking increased translocation frequencies in at least two populations, one in unexposed Americans[22] and the other involving Chernobyl clean up workers[14]. However, the fluctuation in cigarette consumption among smokers in these populations was not estimated and its significance for the purposes of dosimetry is not yet understood.

3.7 Tumor cells are not present in the tissue being analyzed.

Tumor cells frequently have stable chromosome rearrangements, and care must be taken to avoid accidental analysis of these cells in biodosimetry studies. While this is not a major concern, the potential for neoplastic cells must be borne in mind when evaluating translocation frequencies. Due to the clonal nature of tumors, such cells are almost certain to be identified if the study design includes routine examination for clones of cytologically abnormal cells.

3.8 Differences between individuals with respect to the above considerations are negligible, or can be adjusted for.

Historically it has often been assumed that any two people with the same exposure will incur the same amount of damage. This may be generally true for special cases (e.g., radiation shortly after exposure), but in general, individual differences are likely to be very important. The existence of rare genetic disorders (e.g., ataxia telangiectasia) and our rapidly increasing knowledge of polymorphisms in human DNA repair genes[18] precludes categorical disregard for differences in individual susceptibility. Differences in metabolism are well known[3], and an improved understanding of the involvement of specific genes will become increasingly important for individual risk estimation. The association between specific genotypes and chromosome alterations has recently been reviewed[16]. For chemical

exposures in particular, the importance of metabolism upon the induction, persistence and accumulation of genetic damage should not be underestimated.

3.9 Changes in the frequency of genetic damage with age must be well characterized.

The effects of aging have been examined for many genetic endpoints and most show at least small increases with age. The frequency of baseline stable chromosome aberrations has been shown to increase more than 10-fold with age, and age accounted for 70% of the statistical variation between donors[17, 22]. The magnitude of this effect illustrates the importance of understanding the age-dependent aspects of cytogenetic endpoints as well as the need for solid epidemiological designs in population studies.

4 SUMMARY

To the extent that the parameters of induction, accumulation, and persistence are understood, and that the principles for retrospective analysis hold true or can be accounted for, biological dosimetry using chromosome translocations can be achieved many years after exposure. Some factors such as aging and smoking are already known to influence translocation frequencies and these must be carefully considered in any retrospective analysis. The apparent decline in translocation frequencies following exposure, while not uniformly observed, will require particular attention when additional experimental evidence clarifies the conditions under which such declines occur. Improvements in the ability to identify clones of cells with cytogenetic abnormalities is yielding improved dose estimates. The rapidly increasing use of genetic polymorphisms as biomarkers of susceptibility makes it likely that routine genotyping of individuals may aid in the interpretation of translocation frequencies. Genotyping may be especially important when evaluating chemical exposure, as the agent(s) may undergo metabolic conversion to compounds that have greater biological reactivity in some people. Other genetic and environmental factors which influence translocation frequencies may become known as additional work is performed, making the future of retrospective exposure analyses bright and promising.

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